

FIG. 1A

H36.D2.B7 Anti-Tissue Factor Light Chain Variable Region

GACATTCAGATGACCCAGTCTCCTGCCTCCCAGTCTGCATCTCTGGGAGAAAGTGTCCACATCACATGC
D I Q M T Q S P A S Q S A S L G E S V T I T C
CTGGCAAGTCAGACCATTGATACATGGTTAGCATGGTATCAGCAGAAACCAGGGAAATCTCCTCAGCTC
L A S Q T I D T W L A W Y Q Q K P G K S P Q L
CTGATTTATGCTGCCACCAACTTGGCAGATGGGGTCCCATCAAGGTTCAAGTGGCAGTGGATCTGGCACA
L I Y A A T N L A D G V P S R F S G S G S G T
AAATTTTCTTTCAAGATCAGCAGCCTACAGGCTGAAGATTTTGTAATTATTACTGTCAACAAGTTTAC
K F S F K I S S L Q A E D F V N Y Y C Q Q V Y
AGTTCTCCATTACGTTTCGGTGCTGGGACCAAGCTGGAGCTGAAA
S S P F T F G A G T K L E L K

FIG. 1B

H36.D2.B7 Anti-Tissue Factor Heavy Chain Variable Region

GAGATCCAGCTGCAGCAGTCTGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGCAGGTATCCTGCAAG
E I Q L Q Q S G P E L V K P G A S V Q V S C K
ACTTCTGGTTACTCATTCACTGACTACAACGTGTACTGGGTGAGGCAGAGCCATGGAAAGAGCCTTGAG
T S G Y S F T D Y N V Y W V R Q S H G K S L E
TGGATTGGATATATTGATCCTTACAATGGTATTACTATCTACGACCAGAACTTCAAGGGCAAGGCCACA
W I G Y I D P Y N G I T I Y D Q N F K G K A T
TTGACTGTTGACAAGTCTTCCACCACAGCCTTCATGCATCTCAACAGCCTGACATCTGACGACTCTGCA
L T V D K S S T T A F M H L N S L T S D D S A
GTTTATTTCTGTGCAAGAGATGTGACTACGGCCCTTGACTTCTGGGGCCAAGGCACCACTCTCACAGTC
V Y F C A R D V T T A L D F W G Q G T T L T V
TCCTCA
S S

* CDR regions underlined.

0990586-112101

Antibody	Apparent K_d , M^{-1}	Apparent K_d , M
By ELISA		
D2	5.2×10^9	1.9×10^{-10}
I47	6.5×10^9	1.5×10^{-10}
K73	9.8×10^9	1.0×10^{-10}
K80	2.3×10^9	4.3×10^{-10}
L102	2.5×10^9	4.0×10^{-10}
L133	1.7×10^9	5.9×10^{-10}
By BLACore		
<u>H36</u>	<u>3.1×10^{10}</u>	<u>3.2×10^{-11}</u>
I43	2.3×10^9	<u>4.3×10^{-10}</u>
I47	3.2×10^9	<u>3.1×10^{-10}</u>
L133	4.6×10^9	<u>2.2×10^{-10}</u>
M107	1.1×10^9	<u>9.1×10^{-10}</u>

FIG. 2

0990586-12101

Antibody Name	% Inhibition
	Antibody Preincubated with TF/VIIa
D1	0
D1B	1
H31	4
<u>H36</u>	<u>95</u>
I43	1
J131	7
K80	0
K82	0
K87	1
L97B	7
L101	0
L102	0
L105	0
L133	0
M5	1
M107	34

FIG. 3

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Antibody Name	<u>% Inhibition</u> TF Preincubated with Antibody Prior to Addition of VIIa	<u>% Inhibition</u> TF Preincubated with VIIa Prior to Addition of Antibody
D1	15	nd
D1B	48	12.7
H31	64	21
H36	0	0
I43	68	55
J131	38	11
K80	12	nd
K82	0	nd
K87	0	nd
L96	0	nd
L101	38	11
L102	14	nd
L105	4	nd
L133	13	nd
M5	0	nd
M107	0	nd

FIG. 4

[rhTF], nM	[H36.D2], nM	H36.D2/rhTF Molar Ratio	Clotting Time (seconds)	% Inhibition of rhTF Function
0.0048	0	0	102.3	0
	1.61	335.4	114.3	31.3
	3.23	670.8	121.3	45.8
0.023	0	0	77.6	0
	1.61	70.0	85.3	52.2
	3.23	140.0	91.1	65.2
	6.45	280.4	99.6	73.9
0.092	0	0	49.3	0
	3.23	35.1	65.8	65.2
	6.45	70.1	88.5	90.2
	12.90	140.2	113.3	95.7
0.46	0	0	32.6	0
	6.45	14.0	52.7	82.4
	12.90	28.0	80.2	96.7
	32.30	70.2	117.9	99.3
2.30	0	0	23.9	0
	16.10	7.0	47.1	94.4
	32.30	14.0	95.2	99.7
	64.50	28.0	115.3	99.9
11.52	0	0	22.2	0
	16.10	1.4	30.2	93.4
	32.30	2.8	46.0	98.8
	64.50	5.6	87.6	99.9
	161.30	14.0	114.0	100.0

FIG. 5

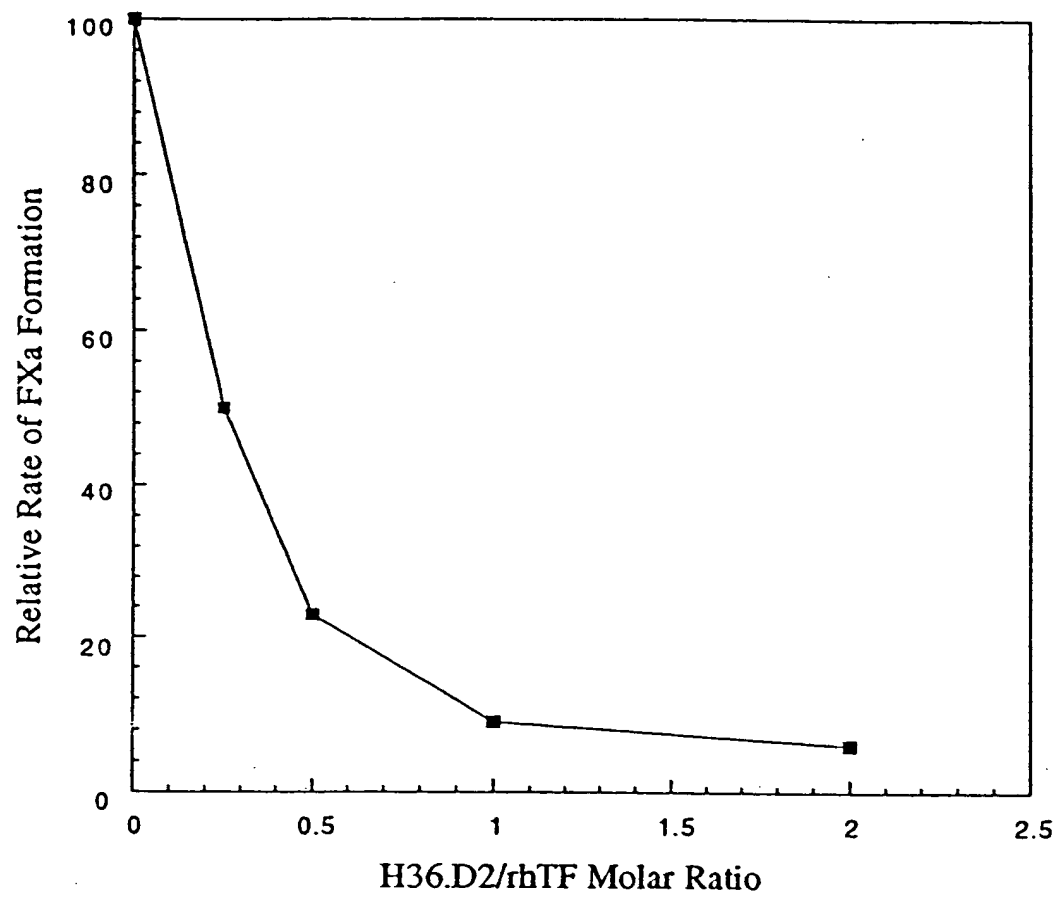


FIG. 6A

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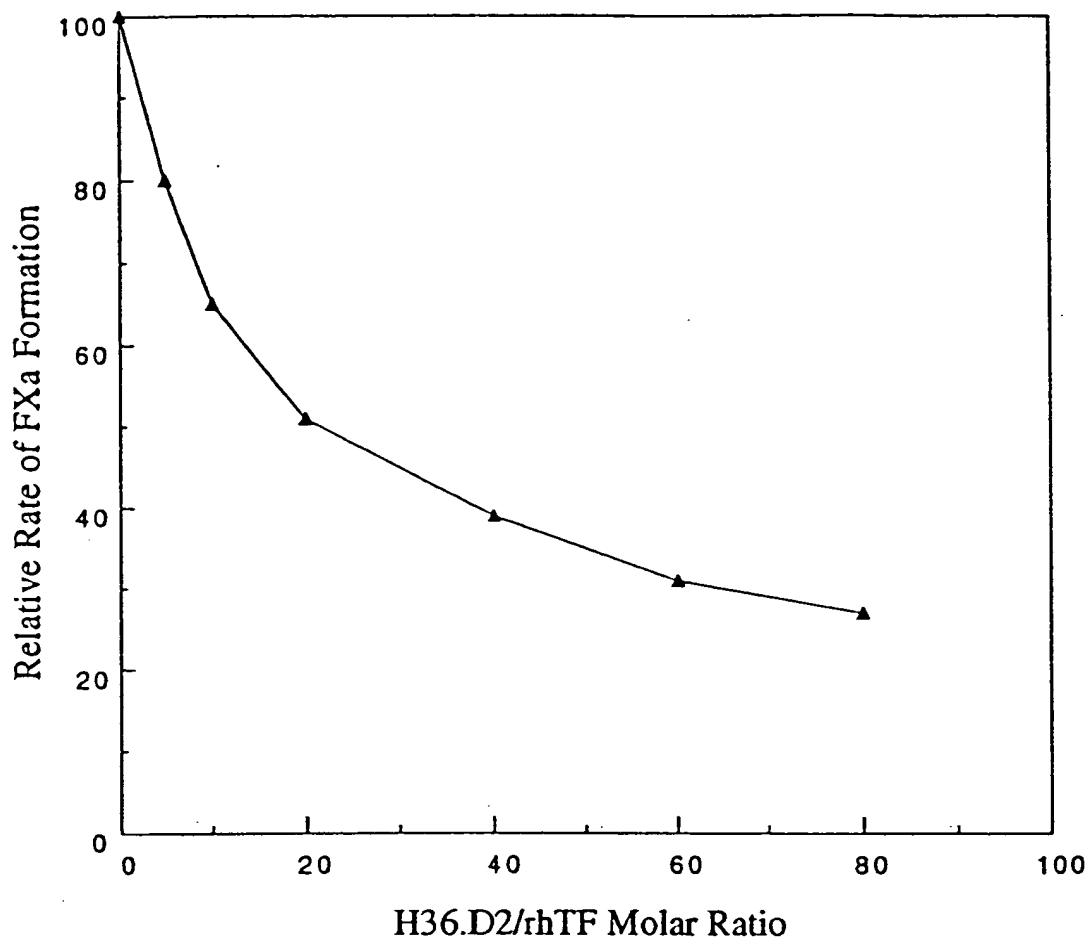


FIG. 6B

H36.D2 Concentration (ng)	<u>% Inhibition</u> Cells (TF/FVII) and H36.D2 preincubated prior to FX addition	<u>% Inhibition</u> FX and H36.D2 are added simultaneously to Cells (TF/FVII)
0	0	0
50	88	nd
100	92	nd
200	97	nd
800	nd	76
1600	nd	78
3200	nd	92

FIG. 7

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FOOTPRINT - 98506660

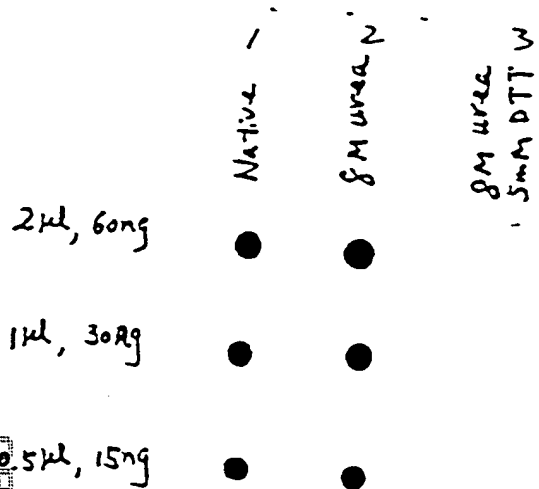


FIG. 8A

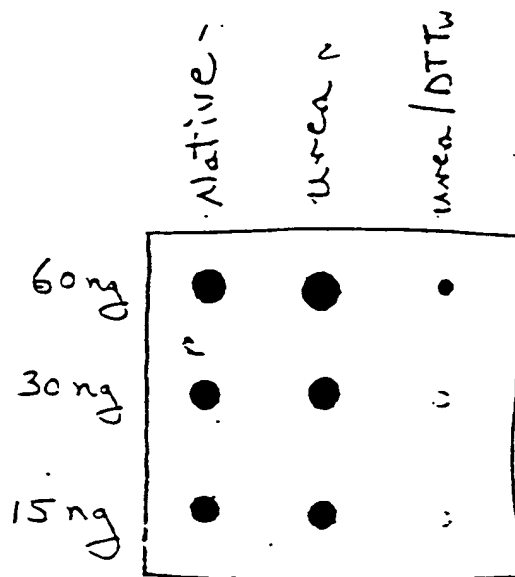
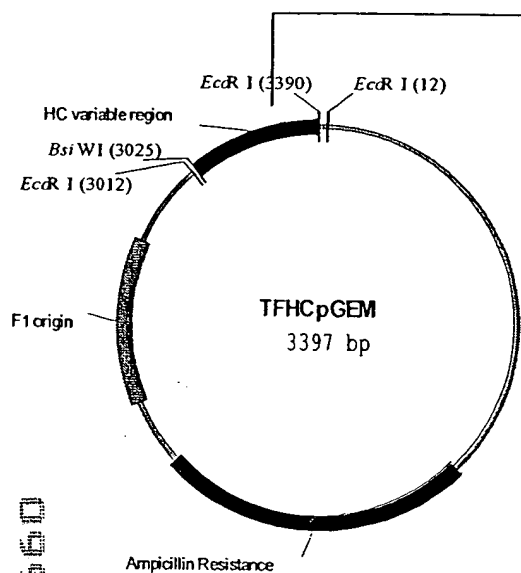


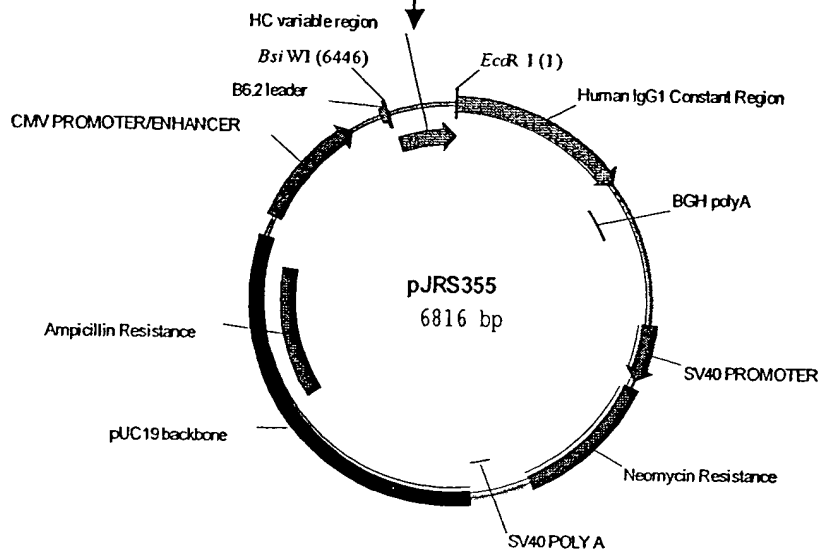
FIG. 8B

Figure A. Human IgG1- χ H36 HC Variable Region Cloning and Expression Vector



HC Cloning Vector

Fig. 9A



HC Expression Vector

Fig. 9B

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Figure B. Human IgG4-cH36 HC Variable Region Cloning and Expression Vector

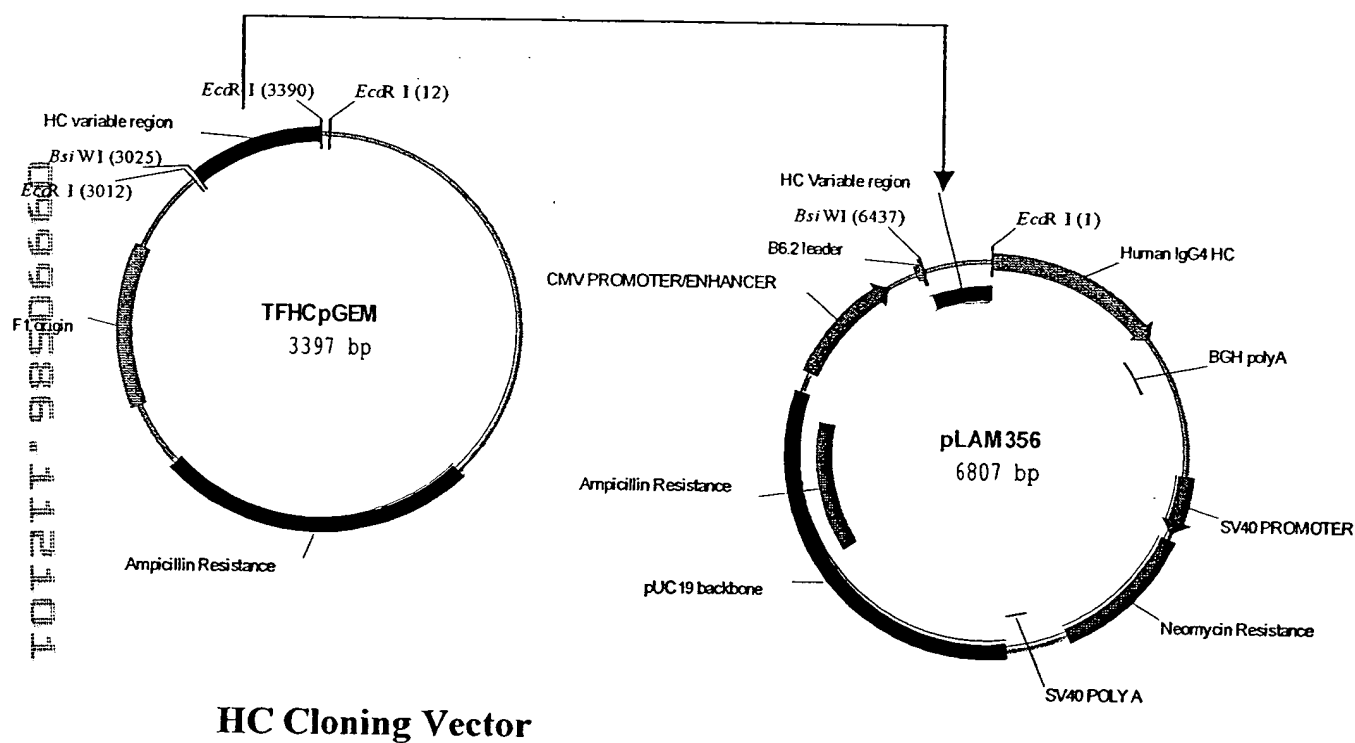


Fig. 9C

HC Expression Vector

Fig. 9D

Figure C. cH36 LC Variable Region Cloning and Expression Vector

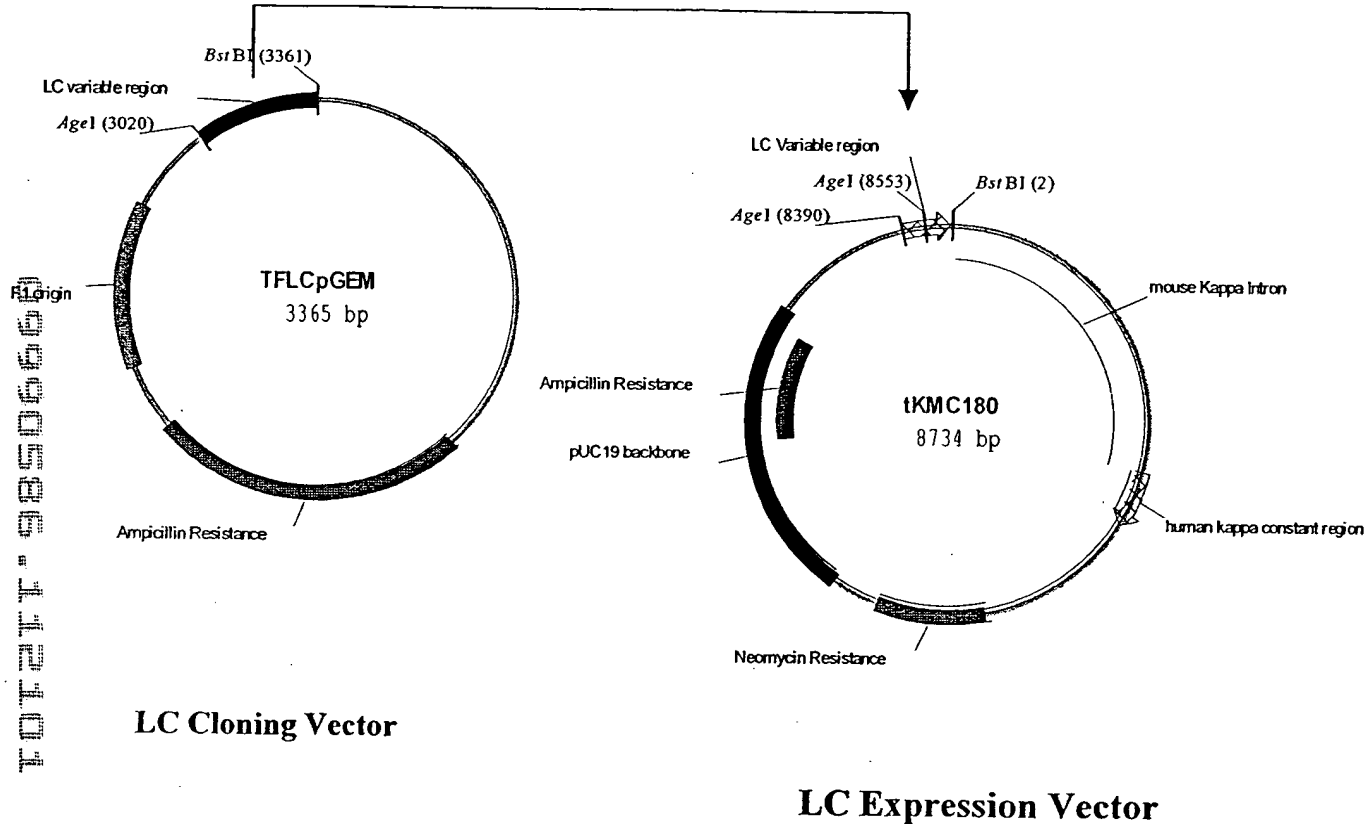


Fig. 10A

Fig. 10B

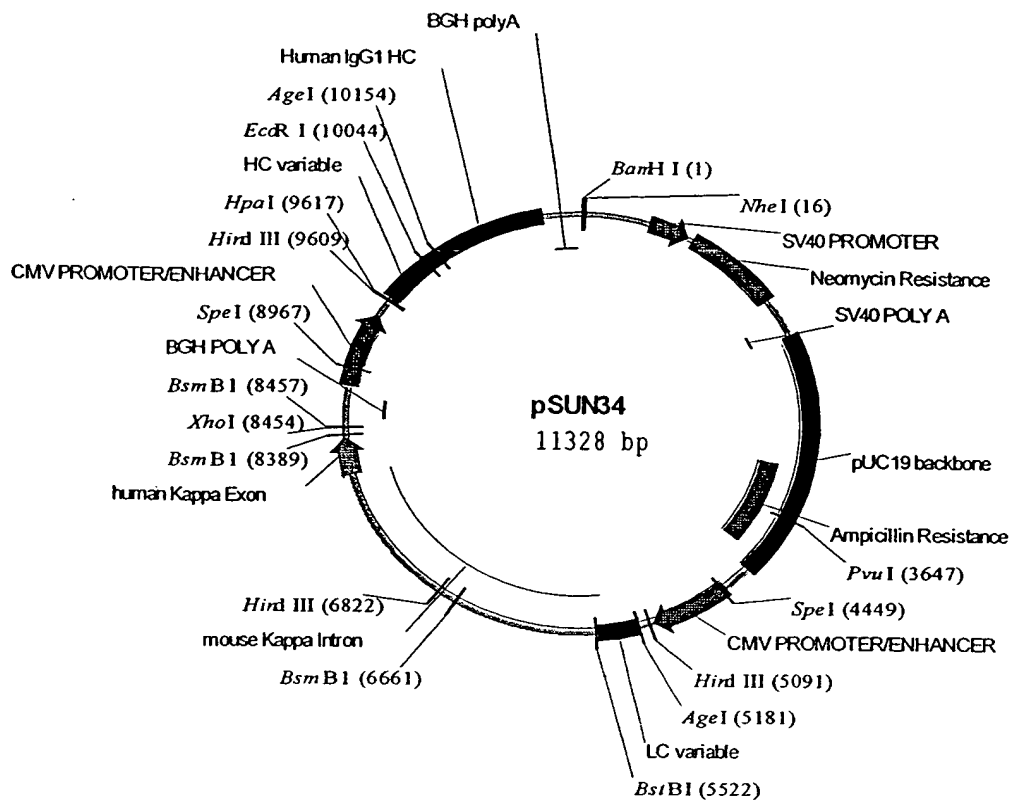


Figure D. Plasmid Map of Humanized Anti-TF IgG1 Antibody Expression Vector

Fig. 11

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Humanization of anti-Tissue Factor Antibody cH36

Sequences of Partially and Fully Humanized Light Chain (LC) Variable Regions

Light Chain (LC) FR Sequences

FR1 (23 AA)	FR2 (14 AA)	FR3 (32 AA)	FR4 (10 AA)	Names
1 10 20 35 47 57 60 70 80 86 98 107				
DIQMTQSPASQASLGEVTITC	WYQKPGKSPQLIY	GVPSRFGSGSGTKFSFKISSLQAEDEVNYYC	FGAGTKLELK	CH36-LC
DIQMTQSPASQASLGEVTITC	WYQKPGKSPQLIY	GVPSRFGSGSGTKFSFKISSLQAEDEVNYYC	FGAGTKLELK	LC-03
DIQMTQSPASQASLGEVTITC	WYQKPGKSPQLIY	GVPSRFGSGSGTKFSFKISSLQAEDEVNYYC	FGAGTKLELK	LC-04
DIQMTQSPASQASLGEVTITC	WYQKPGKSPQLIY	GVPSRFGSGSGTKFSFKISSLQAEDEVNYYC	FGAGTKLELK	LC-05
DIQMTQSPASQASLGEVTITC	WYQKPGKSPQLIY	GVPSRFGSGSGTKFSFKISSLQAEDEVNYYC	FGAGTKLELK	LC-06
DIQMTQSPASQASLGEVTITC	WYQKPGKSPQLIY	GVPSRFGSGSGTKFSFKISSLQAEDEVNYYC	FGAGTKLELK	LC-07
DIQMTQSPASQASLGEVTITC	WYQKPGKSPQLIY	GVPSRFGSGSGTKFSFKISSLQAEDEVNYYC	FGAGTKLELK	LC-08
DIQMTQSPASQASLGEVTITC	WYQKPGKSPQLIY	GVPSRFGSGSGTKFSFKISSLQAEDEVNYYC	FGAGTKLELK	LC-09
DIQMTQSPASQASLGEVTITC	WYQKPGKSPQLIY	GVPSRFGSGSGTKFSFKISSLQAEDEVNYYC	FGAGTKLELK	LC-10
DIQMTQSPASQASLGEVTITC	WYQKPGKSPQLIY	GVPSRFGSGSGTKFSFKISSLQAEDEVNYYC	FGAGTKLELK	LC-11
DIQMTQSPASQASLGEVTITC	WYQKPGKSPQLIY	GVPSRFGSGSGTKFSFKISSLQAEDEVNYYC	FGAGTKLELK	LC-12

Fig. 12A

Light Chain CDR Sequences of cH36

CDR1 (11 AA)	CDR2 (7 AA)	CDR3 (9 AA)
24 34 50 56 97		
L A S Q T I D T W L A	A A T N L A D	Q Q V Y S S P F T

Fig. 12B

Fig. 12C

Fig. 12D

Sequences of Partially and Fully Humanized Heavy Chain (LC) Variable Regions

Heavy Chain (HC) FR Sequences

FR1 (30 AA)	FR2 (14 AA)	FR3 (32 AA)	FR4 (11 AA)	Names
1 10 20 29 36 44 67 75 85 95 107 117				
EQIQQSGPELVKPGASVQVSCKTSGYST	WVRQSHGKSLEWIG	KATLTVDKSSTTAFMHLNLSLTSDSAVYFCAR	WGQGTTLTVSS	CH36-HC
EQIQQSGPELVKPGASVQVSCKTSGYST	WVRQSHGKSLEWIG	KATLTVDKSSTTAFMHLNLSLTSDSAVYFCAR	WGQGTTLTVSS	HC-01
EQIQQSGPELVKPGASVQVSCKTSGYST	WVRQSGKGLLEWIG	KATLTVDKSSTTAFMHLNLSLTSDSAVYFCAR	WGQGTTLTVSS	HC-02
EQIQQSGPELVKPGASVQVSCKTSGYST	WVRQSGKGLLEWIG	KATLTVDKSSTTAFMHLNLSLTSDSAVYFCAR	WGQGTTLTVSS	HC-03
EQIQQSGPELVKPGASVQVSCKTSGYST	WVRQSGKGLLEWIG	KATLTVDKSSTTAFMHLNLSLTSDSAVYFCAR	WGQGTTLTVSS	HC-04
EQIQQSGPELVKPGASVQVSCKTSGYST	WVRQSGKGLLEWIG	KATLTVDKSSTTAFMHLNLSLTSDSAVYFCAR	WGQGTTLTVSS	HC-05
EQIQQSGPELVKPGASVQVSCKTSGYST	WVRQSGKGLLEWIG	KATLTVDKSSTTAFMHLNLSLTSDSAVYFCAR	WGQGTTLTVSS	HC-06
EQIQQSGPELVKPGASVQVSCKTSGYST	WVRQSGKGLLEWIG	KATLTVDKSSTTAFMHLNLSLTSDSAVYFCAR	WGQGTTLTVSS	HC-07
EQIQQSGPELVKPGASVQVSCKTSGYST	WVRQSGKGLLEWIG	KATLTVDKSSTTAFMHLNLSLTSDSAVYFCAR	WGQGTTLTVSS	HC-08
EQIQQSGPELVKPGASVQVSCKTSGYST	WVRQSGKGLLEWIG	KATLTVDKSSTTAFMHLNLSLTSDSAVYFCAR	WGQGTTLTVSS	HC-08R1
EQIQQSGPELVKPGASVQVSCKTSGYST	WVRQSGKGLLEWIG	KATLTVDKSSTTAFMHLNLSLTSDSAVYFCAR	WGQGTTLTVSS	HC-11
EQIQQSGPELVKPGASVQVSCKTSGYST	WVRQSGKGLLEWIG	KATLTVDKSSTTAFMHLNLSLTSDSAVYFCAR	WGQGTTLTVSS	HC-12
EQIQQSGPELVKPGASVQVSCKTSGYST	WVRQSGKGLLEWIG	KATLTVDKSSTTAFMHLNLSLTSDSAVYFCAR	WGQGTTLTVSS	HC-09
EQIQQSGPELVKPGASVQVSCKTSGYST	WVRQSGKGLLEWIG	KATLTVDKSSTTAFMHLNLSLTSDSAVYFCAR	WGQGTTLTVSS	HC-10

f-6.
13A

Heavy Chain CDR Sequences

CDR1 (5 AA)	CDR2 (17 AA)	CDR3 (8AA)	Names
31 35 50			
D Y N V Y	Y I D P Y N G I T I Y D Q N F K G	D V T T A L D F	CH36
31 35 50			
D Y N V Y	Y I D P Y N G I T I Y D Q N K G	D V T T A L D F	HC-08

Fig. 13C

Fig. 13D

hOAT (IgG1) Constant regions sequences

Sequences of LC constant:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYLSSTLTLSKADYEKH

Fig. 14A

KVYACEVTHQGLSSPVTKSFNRGEC

Sequences of HC constant:

EFASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYIC

NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV

Fig. 14B

HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYITLPPSRDELTKNQVSLTCL

VKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK

hFAT (IgG4) constant region sequences

Sequences of LC Constant:

RTVAAPSVFIFPPSDEQLKSGTASVVCCLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSLSSLTLSKADYEK

Fig. 15A

HKVYACEVTHQGLSSPVTKSFNRGEC

Sequences of HC constant:

EFASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTY

Fig. 15B

TCNVDPKPSNTKVDKRVESKYGPPCPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGV

EVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPSQEEMTKNQVSL

TCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGK